

METHOD TO PRESELECT THE SEX OF OFFSPRING

This application is a continuation of application Ser. No. 07/349,669, filed May 10, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a method of preselecting the sex of offspring by sorting sperm into X and Y chromosome-bearing sperm based on differences in DNA content.

2. Description of the Prior Art

Gender of animal offspring is important to livestock producers. Because the dairy farmer has little use for most bull calves, the use of sexed semen to produce only females would make milk production more efficient. Swine farmers would produce pork more efficiently if they were able to market only female swine, because females grow faster than males.

In beef cattle and sheep breeds, the male grows at a faster rate than the female and hence is preferred for meat production.

In addition, the ability to specify male or female offspring should shorten the time required for genetic improvements, since desirable traits are often associated with one or the other parent. Planning the sex of cattle offspring is already practiced on a limited basis. This procedure consists of removing embryos from the cow, identifying their potential gender, and re-implanting only those of the desired gender. However, an ability to separate sperm into male-producing and female-producing groups before they are used for artificial insemination could enhance the overall value of offspring produced by embryo transfer.

Every living being has a set of paired chromosomes, which carry all the genetic material necessary to maintain life and also to propagate new life.

All but one pair of chromosomes are called autosomes and carry genes for all the characteristics of the body, such as skin, hair and eye color, mature size, and body characteristics. The remaining pair are called sex chromosomes. They carry the genetic material that specifies gender. One sex chromosome is called X, the other Y.

A sperm from the male or an egg from the female contains one of each pair of autosomes; in addition, in mammals the egg always contains an X chromosome, while the sperm always carries either an X or Y chromosome.

When a sperm and egg unite and the sperm carries the Y chromosome, the offspring is male (XY); however, if the sperm carries an X chromosome when it unites with the egg, the resulting offspring is female (XX).

The only established and measurable difference between X and Y sperm that is known and has been proved to be scientifically valid is their difference in deoxyribonucleic acid (DNA) content. The X chromosome is larger and contains slightly more DNA than does the Y chromosome. The difference in total DNA between X-bearing sperm and Y-bearing sperm is 3.4% in boar, 3.8% in bull, and 4.2% in ram sperm.

The amount of DNA in a sperm cell, as in most normal body cells, is stable. Therefore, the DNA content of individual sperm can be monitored and used to differentiate X- and Y-bearing sperm.

Since the difference in DNA mass in the sex chromosomes of most mammals is the only scientifically validated, measurable difference between X- and Y-bearing sperm, the chromosomal constitution [Moruzzi, J. Reprod. Fertil. 57: 319 (1979)] and/or measurement of DNA mass [Pinkel et al. (1), Science 218: 904 (1982); Pinkel et al. (2), Cytometry 3: 1 (1982); Johnson and Pinkel, Cytometry 7: 268 (1986); Johnson et al. (1), Gam. Res. 16: 1 (1987); Johnson et al. (2), Gam. Res. 17: 203 (1987)] are the only verifiable means other than fertility for determining the sex-producing capability of a population of sperm. The literature describes many physical, biochemical, and functional methods that have purportedly sexed sperm [Amann and Seidel, "Prospects for Sexing Mammalian Sperm," Colorado Assoc. Univ. Press, Boulder (1982)]; several of these methods have been tested for relative DNA content [Pinkel et al., J. Anim. Sci. 60: 1303 (1985); Johnson (1), Theriogenology 29: 265 (1988)]. However, no method has been proven in controlled experiments to actually affect the sex ratio of offspring.

Previous studies have demonstrated that the difference in DNA content between X and Y chromosome-bearing sperm can be repeatedly measured and the sperm sex ratio of a sample of semen predicted [Johnson and Pinkel, supra; Johnson et al. (1), supra; Johnson et al. (2), supra; Johnson (1), supra; Johnson (2), Cytometry, Suppl. 2: 66 (Abstract) (1988)]. Verifiable separation by sorting of X and Y sperm based on DNA content has been accomplished with the vole [Pinkel et al. (1), supra; Johnson, In "Beltsville Symposia in Agricultural Research X," P. C. Augustine, H. D. Danforth, & M. R. Bakst (eds.), Martinus Nijhoff, Boston, pp. 121-134 (1986)] and the chinchilla [Johnson et al. (1), supra]. However, preparation procedures damaged DNA viability. The sorting of sperm nuclei from several mammalian (bull, boar, ram, vole, chinchilla) species into separate X and Y chromosome-bearing populations at purities ranging from 92 to 99% has been accomplished [Johnson and Clarke, Gam. Res. 21: 335 (1988)]. Nuclear decondensation and pronuclear development was demonstrated in hamster eggs that had been microinjected with sorted X- or Y-bearing bull, boar, or ram sperm [Johnson and Clarke, supra].

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method for sorting mammalian sperm into X and Y chromosome fractions based on DNA content.

It is a further object of this invention to teach a method of staining the DNA of mammalian sperm while maintaining viability of the sperm.

It is a further object of this invention to provide a sheath fluid adapted to be used in a cell-sorting apparatus while maintaining viability of sperm cells.

It is a further object of this invention to provide a collecting fluid capable of maintaining the viability of sorted sperm cells.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

DETAILED DESCRIPTION OF THE INVENTION

I have now demonstrated the separation, by flow sorting, of intact, viable X and Y chromosome-bearing rabbit and swine sperm populations based on relative DNA content; surgical insemination of the sorted sperm into does; and the subsequent birth of sexed offspring